Electron microscopy of rhabdomyosarcoma of the orbit
A study of two cases

Arnold J. Kroll, Toichiro Kuwabara, and George M. Howard

Rhabdomyosarcoma is believed to be the most common primary malignant orbital tumor of childhood. A recent review of 55 cases disclosed three histologic types: embryonal (73 per cent), differentiated (11 per cent), and alveolar (16 per cent). The fine structure characteristics of orbital rhabdomyosarcoma have not yet been reported.

Case reports, materials and methods

Case 1. K. L., a 7-year-old white boy, complained of diplopia and was noted to have progressive protrusion of the right eye of 6 weeks' duration.

Examination of the right eye demonstrated proptosis of approximately 4 mm, with displacement of the globe inferiorly and temporally. Limitation of voluntary motion and diplopia were greatest on attempted gaze upward and inward. A firm, oval, nontender mass approximately 2 cm in diameter was palpable in the superonasal orbit. The vision was 20/25+ and the cornea, anterior chamber, iris, and fundus were unremarkable. Confrontation field was full. On examination, the left eye was entirely normal.

There was no significant past medical history and no family history of orbital tumor. The patient had six normal siblings. The general physical examination was unremarkable, and x-ray studies of the orbits, nasal sinuses, chest, and long bones were negative.

Biopsy of the orbital mass demonstrated a malignancy and the orbit was exenterated. At operation the mass was grayish white, firm, smooth in some areas, lobulated in others, and measured 2 by 2 cm. It was firmly attached to the superonasal aspect of the superior rectus muscle, but did not invade the levator palpebrae or orbital bone.

Case 2. M. B., a 5½-year-old white girl, was admitted to the hospital with proptosis of the right eye of 6 weeks' duration.

Eleven months prior to admission, a binocular rectus muscle recession was performed for eso-
Fig. 1. Case 1. Low-power light micrograph of differentiated rhabdomyosarcoma. Large cells with abundant cytoplasm are scattered among smaller spindle-shaped cells. (Hematoxylin and eosin. Obj. magnification ×10, ocular magnification ×10.)

Fig. 2. Case 1. High-power (oil immersion) light micrograph of a differentiated tumor cell showing cross striations. (Wilder's reticulum stain. Obj. magnification ×100, ocular magnification ×10.)
tropia. Six weeks prior to admission, prominence of the right upper lid was noted and an orbital mass was palpated superonasally. The mass rapidly enlarged, and 1½ weeks prior to admission a biopsy of the mass demonstrated a malignancy.

Two siblings aged 13 and 9 were living and well. A maternal grandfather died of "cancer of the kidney."

Examination of the right eye revealed a 3 mm. displacement of the globe temporally and inferiorly. A well-localized soft 2 cm. mass was palpated superiorly and nasally. Examination of the left eye was within normal limits. X-ray studies of the right orbit revealed a soft tissue density but no invasion of bone. The hemoglobin, white blood cell count, and urinalysis were within normal limits.

The orbit was exenterated and the tumor mass was found to measure 2 by 1½ cm. It was soft and yellowish tan and was infiltrating into the orbital soft tissue.

Immediately after exenteration, each specimen was opened and 2 mm. square pieces of tumor were quickly excised from areas remote from extraocular muscle. Fixation was in cold (4° C.) buffered 2 per cent osmium tetroxide (containing 45 mg. per milliliter of sucrose and 0.002 per cent calcium chloride) for 2 hours. The tumor fragments were then dehydrated in a graded series of ethanol, and embedded in Epon 812.5

Sections were cut with a Servall ultramicrotome with glass or diamond knives, mounted on bare copper grids, stained with uranyl acetate, and counterstained with lead at high pH. The specimens were viewed in a JEM—5Y electron microscope. The unused portion of each tumor was fixed in formalin for light microscopy.

Light microscopy

Case 1. There was an irregular arrangement of large cells with abundant eosinophilic cytoplasm scattered among smaller spindle-shaped cells (Fig. 1). A moderate number of the larger cells demonstrated cross striations with Wilder’s stain (Fig. 2). The diagnosis was rhabdomyosarcoma, differentiated type.

Case 2. There was a loose arrangement of stellate and spindle cells with large non-nucleolated nuclei and scanty, poorly staining cytoplasm (Fig. 3). In the tumor periphery were seen several large, bizarre, multinucleated tumor cells. No cells were found to contain cross striations or longitudinal myofibrils. The diagnosis was rhabdomyosarcoma, embryonal type.

---

Fig. 3. Case 2. Low-power light micrograph of embryonal rhabdomyosarcoma. Stellate and spindle cells are loosely arranged and have scanty cytoplasm. (Hematoxylin and eosin. Obj. magnification ×6.3, ocular magnification ×10.)
Fig. 4. For legend see opposite page.
Fig. 5. Case 1. High-power electron micrograph of an area of cytoplasm of a less well-differentiated tumor cell. Actomyosin filaments (f), on close inspection are noted to be of two types: "thick," 150 Å wide, and "thin," 60 Å wide filaments. RNP particles (r) are seen in very close relationship to some filaments, giving the appearance of filament synthesis. ni, mitochondrion. (×21,000, one micron mark.)

Fig. 4. Case 1. Low-power electron micrograph of a less well-differentiated tumor cell. Note the large cell size and cytoplasmic aggregates of actomyosin filaments (f), among which are collections of RNP particles (r). The filaments are cut in longitudinal, oblique, and cross section and do not have cross striations. Mitochondria (m) are plentiful. Extensive deposits of glycogen (g) are present. The nucleus (n) is indented by large cytoplasmic processes (p) which are seen in both longitudinal and cross section.

In the extracellular space are many collagen fibrils (c), alongside of which is a thin portion of a stromal cell (s). (×11,400, one micron mark.)
Fig. 6. Case 1. Low-power electron micrograph of a well-differentiated tumor cell. The aggregates of actomyosin filaments are relatively well ordered, and pathognomonic banding is seen. The dark Z bands are easily visible. Mitochondria (m) are plentiful and the nucleus (n) contains a large dense nucleolus. (×11,700, one micron mark.)
Fig. 7. Case 1. High-power electron micrograph of an area of a well-differentiated tumor cell. The fine structure is similar to that in embryonal skeletal muscle. A, A band; Z, Z band; I, I band; H, H band; M, M band; r, RNP particles; m, mitochondrion. (x 27,900, one micron mark.)
Fig. 8. Case 1. Stromal cell of the tumor. Although fine filaments are visible within the cytoplasm, these do not have the configuration or dimensions of actomyosin.

RNP particles (r) are both free and bound to membranes. In the latter case they are said to be part of the "rough-surfaced" endoplasmic reticulum (er). Mitochondria (m), vesicles (v), glycogen bodies (g), and lysosomes (l) are seen.

Cyttoplasmic processes (p) project from the cell surface. Collagen fibers (c) are seen in the extracellular space. (×15,000, one micron mark.)
Fig. 9. Case 2. Low-power electron micrograph of embrional rhabdomyosarcoma. The plane of section includes two portions of the nucleus (n). Filaments within the cytoplasm do not have the configuration of actomyosin and must be regarded as nonspecific. A large vesicle (v) is seen at the top of the micrograph. m, mitochondria; gc, Golgi complex. (×14,500, one micron mark.)
Fig. 10. Case 2. Portion of a tumor cell. Clusters of RNP particles (r) and nonspecific filaments are scattered within the cytoplasm. Large mitochondria (m) are cut in longitudinal and transverse section. Fluid-filled vesicles (v) have a rough, particulate border. (x22,600, one micron mark.)
Fig. 11. Case 2. Portion of a tumor cell. An unusual nuclear inclusion (ni) may merely be a fingerlike projection of cytoplasm containing small tubules projecting into the nucleus and cut in transverse section. r, RNP particles; v, cytoplasmic vesicle; gc, Golgi complex. (×25,000, one micron mark.)
Electron microscopy

Case 1.

Actinomyosin filaments. The most characteristic finding in the differentiated rhabdomyosarcoma was the presence of cytoplasmic actomyosin. In the less well-differentiated cells of this tumor, the actomyosin appeared as randomly ordered aggregates of filaments measuring 1.0 to 1.5 \( \mu \) long (Fig. 4). These aggregates were oriented in many different planes, being sectioned obliquely, longitudinally, and across in a single section. With higher magnification (Fig. 5), it was seen that there were two types of filaments, "thick" ones (approximately 150 Å wide) and "thin" one (approximately 60 Å wide). No horizontal cross banding of the filaments could be discerned in the cells whose filaments were randomly oriented.

In the well-differentiated cells of this tumor, however, the filament aggregates were arranged in much less random fashion (Fig. 6). Many of them were aligned with long axes roughly parallel to each other and they manifested the characteristic cross banding of actomyosin. Under higher magnification (Fig. 7) the fine details of the cross banding were better seen. There was a dense 0.10 \( \mu \) Z band within a lucent 0.50 \( \mu \) I band, and a long 1.5 \( \mu \) A band bisected by a lighter 0.25 \( \mu \) H band, which in turn contained a dense 0.05 \( \mu \) M band.

Ribonucleoprotein (RNP) particles. In intimate association with most aggregates of actomyosin filaments were collections of round electron-dense particles measuring approximately 150 Å (Figs. 4 to 7). These particles, usually unattached to membranes, and appearing in groups of up to 30, possessed all the morphologic characteristics of RNP particles (also known as ribosomes or Palade particles). Such particles are known to consist largely of RNA and are found in cells engaged in protein synthesis.5 The RNP particles in these cells were most numerous in areas of high concentration of filaments and indeed could be occasionally found actively engaged in what appeared to be filament synthesis (Fig. 5). Some variation in RNP particle size was noted. However, no correlation could be found between the location of different particle sizes and synthesis of different bands of filaments.6

Mitochondria. Mitochondria were distributed throughout the cytoplasm. They were abundant and varied greatly in size, shape, and in numbers and configuration of cristae. Some (Figs. 5 and 6) contained scattered collections of amorphous material. The mitochondria of the primary tumor cells (Figs. 4 to 7), were as a rule larger and were of more variable shape than the mitochondria of stromal cells (Fig. 8).

Stromal cells. A variety of cell types in this tumor contained no actomyosin filaments (Fig. 8). They were stellate or spindle-shaped cells with a single nucleus and many short cytoplasmic processes. Cytoplasmic organelles were in great abundance, with many mitochondria, small and large vesicles, pigment granules, Golgi membranes, lysosomes, fine filamentous strands, and miscellaneous nonspecific cytoplasmic components. The extracellular space contained moderate numbers of collagen fibers and aggregates of amorphous ground substance.

In contrast to the primary tumor cells themselves, where RNP particles were rarely found to be attached to membranes, the stromal cells commonly contained RNP particles attached to membranous structures. The attachment of BNP particles to membranes defines the structure of "rough-surfaced" endoplasmic reticulum.

Case 2. The embryonal rhabdomyosarcoma (Figs. 9 to 11), unlike the differentiated tumor, contained no cytoplasmic filaments having the characteristics of actomyosin. Clusters of free RNP particles were, however, plentiful, as were mitochondria, fluid-filled vesicles, and Golgi membranes. The nuclei were large and nonnucleolated. Most of the tumor cells looked alike. The intercellular matrix was very loosely arranged with only occasional collagen or nonspecific fibrils seen.
Discussion

It was fortunate, for the sake of comparison, to obtain one differentiated rhabdomyosarcoma and one embryonal type. This provided an opportunity to study very well-differentiated and very poorly differentiated tumor cells with the high resolution of electron microscopy.

The differentiated rhabdomyosarcoma exhibited cells whose cytoplasmic actomyosin was in disordered array (Figs. 4 and 5), and cells whose actomyosin was fairly well ordered (Figs. 6 and 7). One interpretation of this might be that the more random the alignment of actomyosin filaments, the more poorly differentiated the cell. Further extension of this hypothesis could lead to the conclusion that the most poorly differentiated tumor cells would not contain any actomyosin at all.

Porterfield and Zimmerman found convincing cross striations in only 60 per cent of embryonal rhabdomyosarcomas. However, Haramoto and associates demonstrated immunohistochemically that 3 out of 3 embryonal tumors had cytoplasmic actomyosin. It was hoped that electron microscopy would demonstrate actomyosin in the embryonal tumor even though none could be demonstrated with light microscopy. However, no characteristic actomyosin was found.

The significance of the “thick” and “thin” actomyosin filaments is worthy of mention. It is presently believed that the actin of muscle tissue is located in the “thin” filaments and that the myosin is present in the “thick” filaments. A recent study of rhabdomyosarcoma with light microscopy compared the cytologic features of the tumor with normal muscle of the human embryo (6 to 13 weeks’ fertilization age). The conclusion was that embryonal rhabdomyosarcoma closely resembled developing muscle in the 7 to 10 week fetus.

Although, to our knowledge, no report of the fine structure of developing muscle in the human embryo has yet been published, there is a recent report of electron microscopy of myogenesis in rat embryos. It was found that the developing myoblast contained “thick” and “thin” myofilaments, cross banding, glycogen deposits, membrane-free ribosomes, increased numbers of polymorphic mitochondria, and smooth-surfaced vesicles.

A study of electron micrographs of the rat myoblast reveals a striking similarity between these normal embryonic cells and the differentiated rhabdomyosarcoma reported above. The very youngest rat myoblasts bear some resemblance to the embryonal rhabdomyosarcoma described herein.

Unlike normal embryonic cells, however, tumor cells do not further differentiate into an adult configuration of functioning tissue. It is apparent that morphologic similarity does not imply functioning similarity. The explanation for this apparently lies in the realm of the biochemist.

We wish to acknowledge the technical assistance of Dr. Michio Oikawa and Mr. Claude Delise. We thank Dr. Virgil Cuten, Dr. Benjamin Sachs, and Dr. Irving Maneull for their cooperation.

REFERENCES

Discussion

Dr. L. E. Zimmerman, Washington, D. C. I was very pleased to have had the opportunity to read and discuss this fine paper by Dr. Kroll and his colleagues. Dr. Kroll was very obliging. He not only sent me his manuscript early, but he permitted me to make sections and special stains of the tumor so that I could be better prepared to criticize his paper. In the original manuscript, the tumor was called an embryonal rhabdomyosarcoma. I was truly impressed by this demonstration that an embryonal rhabdomyosarcoma could produce so many cells with such a high degree of cytoplasmic differentiation as was demonstrated in the authors' beautiful electron micrographs. However, after studying the tumor myself, by light microscopy, it was evident that this was not an embryonal rhabdomyosarcoma, but rather a differentiated rhabdomyosarcoma. Within the first few moments that I began examining this tumor, I had found neoplastic cells with abundant myoplasm and typical cross striations. A very great proportion of the cells of this tumor showed some degree of differentiation. This is in contrast to our experience with embryonal rhabdomyosarcomas. As their name implies, those are very poorly differentiated tumors usually requiring an exhaustive search before acceptable cross striations are found. In fact, one does well to find cross striations in more than half the cases.

In my experience, orbital rhabdomyosarcomas are rarely so well differentiated as was Dr. Kroll's tumor. Among the 55 tumors in the series that Dr. Porterfield and I reported,1 there were only 6 that were labeled as differentiated rhabdomyosarcoma; 40 were considered embryonal and 9 were alveolar. Dr. Kroll was, therefore, fortunate in having had an opportunity to study one of these rare tumors with such a high degree of differentiation. I am glad that in the paper he has just presented he changed his diagnosis to differentiated rhabdomyosarcoma.

Dr. Kroll has recently had the opportunity to study a truly embryonal rhabdomyosarcoma, one in which the tumor cells were so undifferentiated that I could find only a very small percentage with ribbons of myoplasm and none with acceptable cross striations. Dr. Kroll informs me that he, too, was unable to demonstrate actomyosin filaments, even by electron microscopy.

Within the past week I have had an opportunity to see a manuscript submitted for publication by Chapman, Jones, and Speelberg2 who have studied 2 embryonal rhabdomyosarcomas by electron microscopy. In neither of their specimens were they able to identify definite myofibrils within the tumor cells, though intracytoplasmic filaments of some sort were found in at least one of the tumors. The possibility that those might represent primitive myofilaments was suggested by the authors.

It should be pointed out that light and electron microscopy each has its advantages and disadvantages. It is entirely conceivable that, with the better resolution available to him, the electron microscopist might be able to identify actomyosin filaments in a tumor that the light microscopist reports as having no cross striations. On the other hand, the electron microscopist is able to sample a much more minute fraction of the tumor than does the light microscopist. I suspect that if it were possible to make a comparative study, we would find that for diagnostic purposes the electron microscope has no advantage over the light microscope in the study of rhabdomyosarcomas.

The question always arises as to how legitimately a tumor may be considered an embryonal rhabdomyosarcoma if the tumor cells are not observed to produce actomyosin filaments and cross striations. This is a problem that the pathologist deals with constantly. There are melanomas that elaborate no pigment, undifferentiated adenocarcinomas that fail to produce glandular or ductal structures, poorly differentiated squamous cell carcinomas that do not form prickle cells or keratin, and so on. One must do the best he can with what he has. The more material that is studied and the greater the length of time that the sections are scrutinized, the better the chances of successful demonstrations of cross striations. We have had the experience several times, however, of being successful with recurrent or metastatic tumors after having previously been unsuccessful with the primary tumor. The embryonal rhabdomyosarcomas that we have so diagnosed in spite of our failure to demonstrate cross striations are otherwise indistinguishable clinically as well as histologically from those with acceptable cross striations.3

One observation made by Chapman and co-workers2 prompts me to ask one question of Dr. Kroll. They described and illustrated aggregations of dense intranuclear and intracytoplasmic particles which might be a virus. Dr. Kroll, did you encounter any similar viruslike particles in your rhabdomyosarcoma?

---

1 When presented orally, only the first of the two cases was included.
REFERENCES


Dr. Kroll. I would certainly like to thank Dr. Zimmerman for the splendid effort he has made to evaluate our paper objectively. He was not content merely to study our light microscopy slides, but sectioned our paraffin blocks himself. I have found our recent busy correspondence most rewarding.

Probably the most important limitation of the electron microscope in studying tumors is the small sample of tissue which can be evaluated. The techniques of sectioning limit the size of the block face to 1 mm. square. The sections themselves are only 0.1 μ or less in thickness. The number of cells one might inspect within a reasonable period of time is therefore quite limited. However, if, as in the first case reported above, the tumor cells do contain actomyosin, the electron microscope will quickly detect it.

As to viruslike particles in rhabdomyosarcoma, we were unable to detect any in our two tumors. We would be much interested in seeing the electron micrographs which demonstrate them.